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Serum Tumor Markers in Colorectal Cancer Staging, Grading, and Follow-Up

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Early diagnosis of colorectal cancer, a frequent neoplasia in industrialized countries, permits curative surgery. In this study we assessed the clinical role of serum tumor markers determination in diagnosing, staging, and grading colorectal cancer; the role of carcinoembryonic antigen (CEA), CA 19-9, tissue polypeptide antigen (TPA) and CA 72-4 in colorectal cancer follow-up was also assessed. In 114 patients with colorectal cancer, the oncofetal antigen CEA was compared with the membrane-associated glycoproteins CA 19-9, CA 242, and CA 72-4 and with the cytokeratins TPA, tissue polypeptide-specific antigen (TPS) and tissue polypeptide monoclonal antigen (TPM). Overall, the most sensitive indices were TPA and TPS (67% and 70%, respectively). Tumor stage influenced the levels of CEA, CA 19-9, and TPA, but not those of TPS, while tumor grade influenced CEA and TPS, but not CA 72-4, TPA, and TPM. TPA was the most sensitive index in identifying early or well-differentiated colorectal cancers. The sensitivity was enhanced when this marker was determined in combination with CEA, in diagnosing both advanced and early colorectal tumors. Seventy-seven patients were followed up after therapy for at least 18 months. CEA was the most sensitive index of recurrence (58%); however, this sensitivity is too low to consider tumor markers useful in colorectal cancer follow-up.

KEY WORDS: colorectal cancer, CEA, CA 19-9, CA 72-4, TPA

INTRODUCTION

Colorectal cancer is the most common malignancy of the gastrointestinal tract and together with breast and lung cancer is one of the most frequent malignancies of industrialized countries [1]. This neoplasia can be cured surgically if an early diagnosis is made. However, as it has few symptoms, it is often diagnosed when distant metastases are already present. In an attempt to identify this tumor in its early stages, several diagnostic approaches have been used: endoscopic follow-up of high-risk patients and of those with familial polyposis or ulcerative cholitis, screening of occult blood in stools for subjects over 60 years of age, and, last but not least, determination of serum tumor markers [2–12].

The first tumor marker to be introduced to clinical practice, carcinoembryonic antigen (CEA), has good sensitivity and specificity for the diagnosis of colorectal cancer [5,6,8,10,12], giving the best results in colorectal cancer follow-up after therapy [13–20]. However, CEA alone is not sufficient for an early diagnosis [3,5,6,10,12,14,17,18]. To overcome the limitations of CEA, several other tumor markers have been proposed

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for the diagnosis not only of colorectal, but also of other gastrointestinal tumors [4–6,8–10,12–14,16,17,20].

Three main classes of tumor markers may be identified: members of the immunoglobulin superfamily, such as CEA; membrane-associated glycoproteins, such as CA 19-9, CA 242, and CA 72-4; and cytokeratins, such as tissue polypeptide antigen (TPA). Members of the immunoglobulin superfamily include, in addition to CEA, another molecule whose corresponding gene is known as deleted in colorectal carcinoma (DCC), which is known to contribute to the progression of colorectal tumorigenesis [21]. Membrane glycoproteins are involved in cell–cell interactions [22,23]. Cytokeratins form the intermediate cytoskeleton of epithelial cells [24] and increase in the circulation in the presence of rapid cell growth. TPA was the first cytokeratin to be studied [25]. The antibody elicited against tissue polypeptide-specific antigen (TPS) recognizes the M3 epitope of the TPA molecule; tissue polypeptide monoclonal (TPM) antigen is recognized by three monoclonal antibodies directed against three different epitopes of the TPA molecule [26].

The aims of our study were to assess the clinical value of the oncofetal antigen CEA, the glycoprotein markers CA 19-9, CA 242, CA 72-4, and the cytokeratins TPA, TPS, and TPM alone and in combinations in the diagnosis of colorectal cancer; we also assessed the influence of tumor grade and stage on the circulating levels of these markers. The role of CEA, CA 19-9, TPA, and CA 72-4 in monitoring colorectal cancer recurrence after surgery were also evaluated.

MATERIALS AND METHODS

We studied a total of 114 patients (78 males, 36 females, age range 27–82). All had colorectal cancer, histologically diagnosed from biopsies taken during surgery. Ninety patients underwent radical treatment and 24 palliative surgery. Tumor stage was classified on the basis of surgical and tomographic findings, as follows: stage 1, tumors limited to the mucosa (n = 50); stage 2, tumors involving the muscolaris mucosae (n = 13); stage 3, tumors involving the serosal surface (n = 33); and stage 4, tumors with lymphnode and/or distant metastases (n = 18) [27]. Tumor grade was established by two independent pathologists, with three categories identified: well (G1, n = 19), moderate (G2, n = 85) and poorly (G3, n = 7) differentiated.

Of the 114 patients, 77 entered a follow-up protocol, based on clinical, radiological (liver ultrasonography, thorax radiography), and biochemical (CEA, CA 19-9, TPA, and CA 72-4) monitoring performed every 3 months. Table I reports the clinical characteristics of these 77 patients.

In the sera of all subjects, the following tumor markers were assayed: CEA (Sorin Biomedica, Saluggia, Italy), CA 72-4 (Centocor, Malvern, PA, USA), TPA and TPM (Byk Gulden, Milan, Italy), and TPS (Beki Diagnostic, Bromma, Sweden), measured by means of an immunoradiometric (IRMA) method; CA 19-9 (Byk Gulden, Milan, Italy) by means of an immunoluminometric (ILMA) method; and CA 242 (Pharmacia, Milan, Italy) by means of an immunofluorimetric (IFMA) procedure.

The statistical analysis was done using one-way analysis of variance (ANOVA), Bonferroni's test for pairwise comparisons, Kruskal–Wallis test, and Student's *t*-test.

TABLE I. Colorectal Cancer Patients Subjected to Follow-up*

No. of cases	Surgery	Recurrence	Follow-up (mo) Mean	Range
16	Palliative	Yes	11	6–18
49	Curative	No	24	18–36
12	Curative	Yes	13	6–18

*Patients have been subdivided according to (1) type of surgery, and (2) detection of recurrent disease during follow-up.

TABLE II. Number of Patients With Colorectal Cancer and High Levels of CEA, CA 19-9, CA 242, CA 72-4, TPA, TPS, and TPM

	No. of positive cases/total cases	Sensitivity
CEA >5 μg/L	42/114	37
CA 19-9 >37 U/ml	20/113	18
CA 242 >30 U/ml	16/78	20
CA 72-4 >4 U/ml	29/114	25
TPA >55 U/L	76/114	67
TPS >80 U/L	36/51	70
TPM >46 U/L	52/89	58

TPA, tissue polypeptide antigen; TPS, tissue polypeptide-specific antigen; TPM, tissue polypeptide monoclonal antigen.

RESULTS

Table II shows the number of patients with colorectal cancer having high levels of serum tumor markers. The cutoff values were chosen according to Barone et al. [3] for CEA, Ohuchi et al. [4] for CA 72-4, Huber et al. [8] for CA 19-9, and according to Plebani et al. [28] for CA 242. The limits of TPA, TPS, and TPM were calculated by means of mean +2 SD of values from a control population of blood donors (n = 100 cases).

Figure 1 shows the behavior of CEA, CA 19-9, and TPA in colorectal cancer patients subdivided according to tumor stage; the results of a statistical analysis are also reported. CA 242 and TPS did not vary in relation to tumor stage ($F = 2.40$, *P:* ns, and $F = 2.12$, *P:* ns, respectively). CA 72-4 and TPM levels were significantly higher in patients with advanced tumors (stage 4) than in those

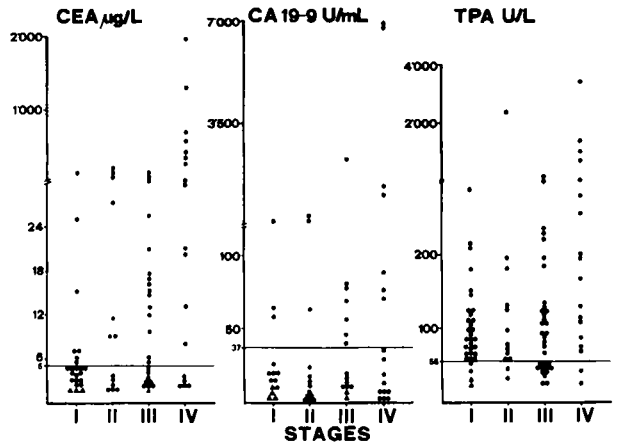

Fig. 1. Individual values of CEA, CA 19-9, and TPA in colorectal cancer patients subdivided according to tumor stage. CEA, CA 19-9, and TPA mean values were significantly higher in stage IV as compared to stage I, II, and III patients (one-way ANOVA: F = 8.15, $P < 0.001$; F = 5.24, $P < 0.005$; and F = 5.23, $P < 0.005$). One triangle = 10 cases.

TABLE III. Number of Positive Cases and Sensitivity (in Parentheses) of CEA, CA 72-4, TPA, and CA 19-9 and of Their Combined Use in Diagnosing Colorectal Cancer in Patients Subdivided According to Tumor Stage

	Stage			
	I (n = 50)	II (n = 13)	III (n = 33)	IV (n = 18)
CEA	7 (14%)	7 (54%)	15 (45%)	13 (72%)
CA 72-4	7 (14%)	4 (31%)	10 (30%)	8 (44%)
TPA	28 (56%)	11 (85%)	21 (64%)	16 (89%)
CA 19-9	3 (6%)	3 (23%)	7 (21%)	7 (39%)
CEA or CA 72-4	13 (26%)	10 (77%)	19 (57%)	15 (83%)
CEA or TPA	32 (64%)	12 (92%)	26 (79%)	17 (94%)
CEA or CA 19-9	10 (20%)	8 (61%)	17 (51%)	15 (83%)

with stage 1, 2, and 3 tumors (one-way ANOVA: F = 3.34, $P < 0.05$, and F = 4.36, $P < 0.01$).

Only CA 19-9 levels were related to tumor grade (Kruskal–Wallis test: H = 8.75, $P < 0.05$), higher in G3 than in G1 and G2 patients.

Tables III and IV report on the sensitivity of CEA, CA 19-9, CA 72-4, and TPA in colorectal cancer staging and grading. The results of their combined use are also given.

Figure 2 illustrates mean values +SEM of CEA, CA 19-9, TPA, and CA 72-4 measured every 3 months in the 49 patients subjected to curative tumor resection and with no signs of recurrence during follow-up. Figure 3 shows the same markers during follow-up evaluation of patients subjected only to palliative surgery. Figure 4 shows the percentage increase of CEA levels with respect to postsurgical individual values in patients with recurrence. Patients with local (n = 3 cases), hepatic (n = 5), and pulmonary (n = 4) metastases have been identified. Both

TABLE IV. Number of Positive Cases and Sensitivity (in Parentheses) of CEA, CA 72-4, TPA, and CA 19-9 and of Their Combined Use in Diagnosing Colorectal Cancer in Patients Subdivided According to Tumor Grade

	Tumor grade		
	G1 (n = 19)	G2 (n = 85)	G3 (n = 7)
CEA	5 (26%)	30 (35%)	4 (57%)
CA 72-4	5 (26%)	19 (22%)	4 (57%)
TPA	12 (63%)	55 (65%)	6 (86%)
CA 19-9	1 (5%)	13 (15%)	5 (71%)
CEA or CA 72-4	7 (37%)	43 (50%)	6 (86%)
CEA or TPA	14 (74%)	69 (81%)	6 (86%)
CEA or CA 19-9	6 (31%)	38 (45%)	6 (86%)

CA 19-9 and TPA increased in the three patients with local recurrence; CA 19-9 increased in one of five and in one of four, while TPA increased in three of five and in one of four patients with hepatic and pulmonary metastases, respectively. The maximal percentage increase noted in patients with no recurrence significantly differs from that found in patients with recurrence only for CEA (Student's *t*-test: t = 1.97, $P < 0.05$ for CEA; t = 0.85, *P:* ns for CA 19-9; t = 1.18, *P:* ns for TPA; and t = 0.66, *P:* ns for CA 72-4).

DISCUSSION

The use of CEA serum determination in diagnosing and monitoring colorectal cancer is widely accepted [3,5–8,10,12–20]. However, while it is suggested to be helpful in postsurgical follow-up evaluation, it is of limited sensitivity and specificity for diagnostic purposes. To overcome these drawbacks, other tumor markers have been proposed in association with CEA, including CA 19-9, CA 50, and CA 72-4 [4,5,8–10,12–14,16,17,20].

We have ascertained the clinical value of seven different tumor markers in diagnosing colorectal cancer as a first step of a project intended to improve the clinical monitoring and follow-up of patients with colorectal carcinoma. When patients were considered altogether, the most sensitive indices were the cytokeratin markers TPA, TPS, and TPM, which gave overlapping results. This finding probably reflects the nature of these substances, which increase in the sera when rapid cell growth occurs [25,29]. However, this phenomenon is nonspecific, since not only different tumors, but also benign proliferative phenomena, may increase their release in the circulation [30–32]. The glycoprotein markers CA 19-9, CA 242, and CA 72-4 gave positive results in only a few patients and, as their sensitivity never exceeded 30%, they cannot be proposed for the diagnosis of colorectal cancer. CEA had an overall sensitivity of 34%, which is slightly lower than reported elsewhere. This discrepancy may be attributable to the fact that only 16% of our patients had ad-

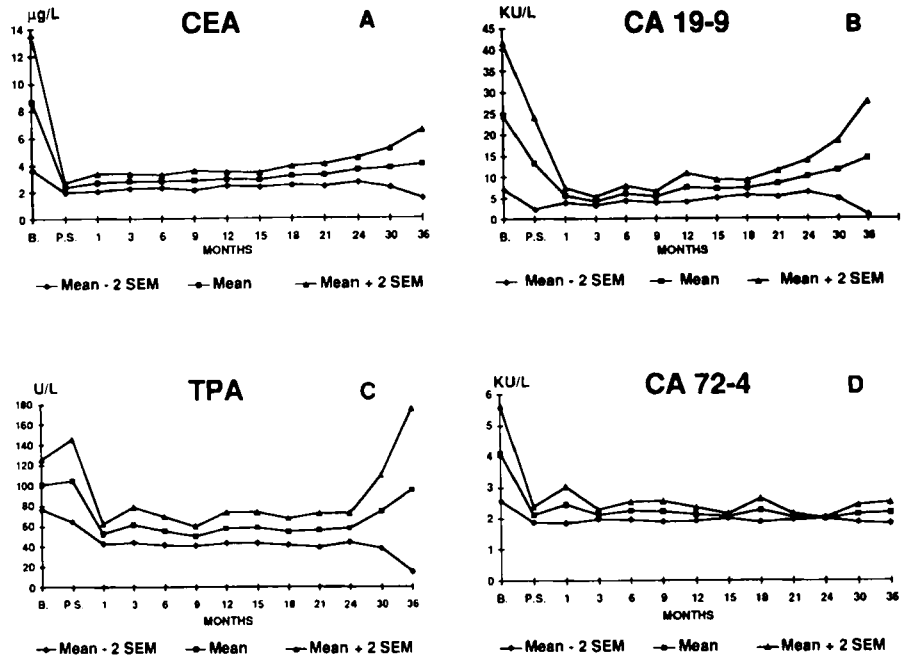

Fig. 2. Mean values ±SEM of CEA, CA 19-9, TPA, and CA 72-4 found during follow-up of patients with no recurrence after surgery. B., preoperative basal values; P.S., postoperative values.

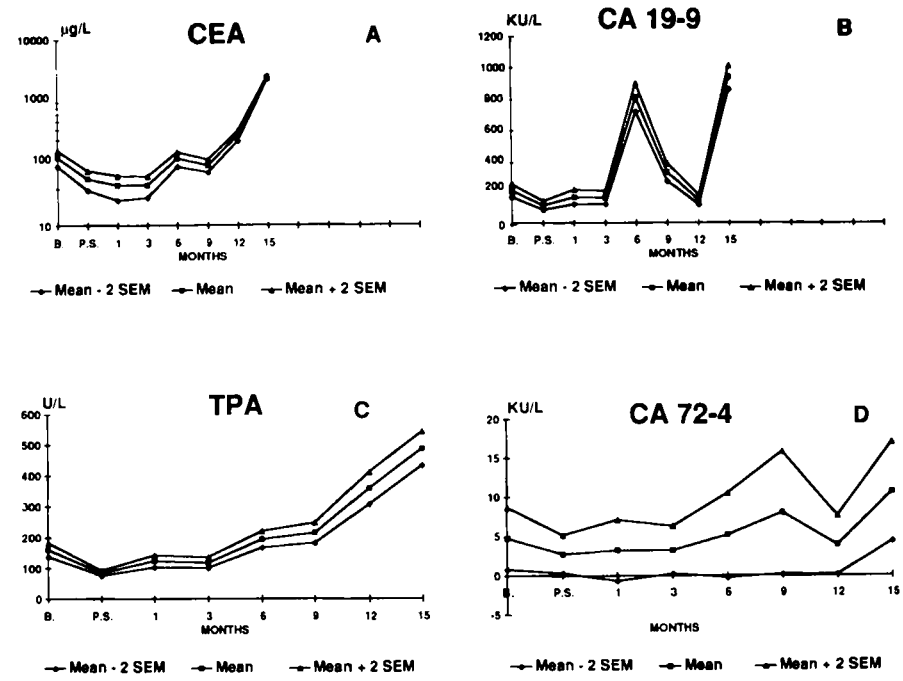

Fig. 3. Mean values ±SEM of CEA, CA 19-9, TPA, and CA 72-4 found during follow-up of patients subjected to palliative surgery. B., preoperative basal values; P.S., postoperative values.

vanced disease at sampling. Our findings, in fact, confirm that CEA increases parallel tumor spread, with the highest values recorded in patients with metastases. Similar behaviors were found for CA 72-4, CA 19-9, TPA, and TPM, whereas TPS and CA 242 levels were not influenced by tumor stage. In particular, high levels of TPS and CA 242 were recorded in 46% and 13% stage 1 patients, respectively. This finding suggests that TPS might be a

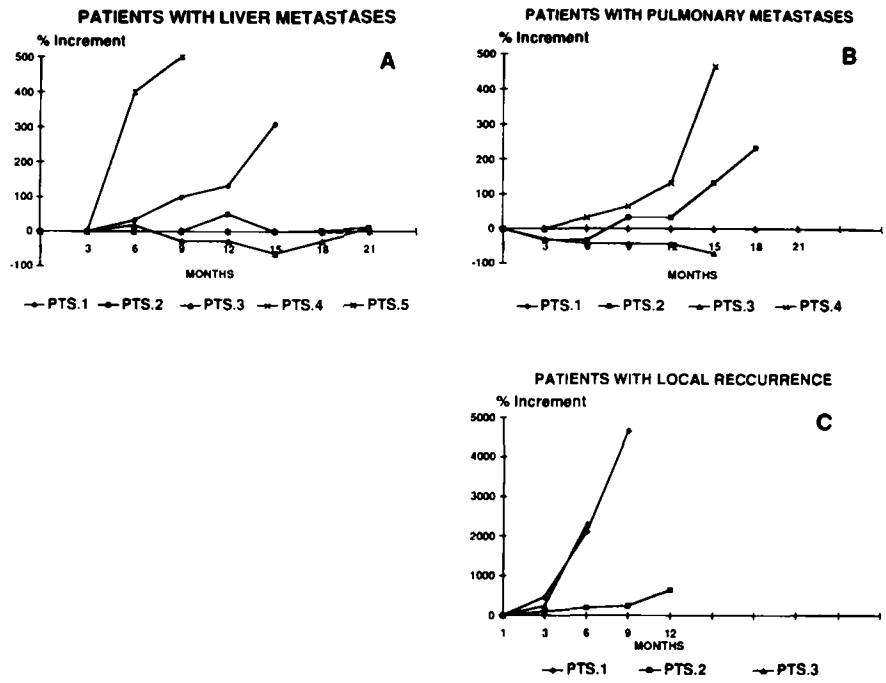

Fig. 4. Percentage of CEA increase in patients with recurrence. Each line represents a single patient; the end of each line corresponds to the time when the diagnosis of recurrence was made. Five patients (PTS.1 to PTS.5) had liver metastases; another group of four patients (PTS.1 to PTS.4) had pulmonary metastases, while three others (PTS.1 to PTS.3) had local recurrence.

useful indicator of early colorectal cancer, as already found for pancreatic cancer [32].

In assessing colorectal cancer, it is important to evaluate both tumor stage and grade, as patients with undifferentiated tumors have a poorer prognosis. With this in mind, we checked for any association between tumor grade and tumor marker levels. Only CA 19-9 and CA 242 reflected tumor grade, with the highest levels found in the more anaplastic tumors.

Because of the limited diagnostic value of the evaluation of single markers, especially in the early stages, we assessed the diagnostic value of the combined use of three markers. We chose CEA because of its specificity toward colorectal carcinoma, CA 72-4 and CA 19-9 as the most sensitive among glycoprotein tumor markers, and TPA as a cytokeratin marker. Combined evaluation of TPA and CEA had the greatest sensitivity in diagnosing not only advanced or poorly differentiated, but also localized and well-differentiated, colorectal tumors.

In conclusion, the diagnostic flowchart of colorectal cancer may include CEA and TPA serum determination, since this combination may have some advantages, especially in identifying colorectal cancer in its early stages. The additional use of other serum markers is not recommended, as these markers provide no more information than that already obtained with CEA and TPA assays.

So far, CEA is considered a good index of colorectal cancer recurrence and is usually used in follow-up. In this study, we evaluated its role, in comparison with the role of CA 19-9, TPA, and CA 72-4, in predicting tumor recurrence. Forty-nine patients followed up for at least 18 months showed no sign of recurrence. In these patients, the four markers behaved similarly, decreasing soon after surgery and remaining almost constant throughout follow-up. By contrast, the 16 patients with persistent disease had levels that increased progressively, probably paralleling tumor growth. Very disappointing data were observed in patients with a recurrent disease. CEA increased significantly in two of five, in two of four, and in three of three patients with hepatic, pulmonary, and local recurrence, respectively, and the degree of this increment was significant in comparison with that found in patients with no recurrence. The other three markers were less sensitive than CEA in predicting recurrence; the degree of their increment did not differ significantly from that found in patients with no recurrence. These data point out two main considerations: (1) the lack of tumor marker increase during follow-up does not mean that the patient is free of recurrence; and (2) tumor marker increases up to 400% over postoperative basal levels were found not only in patients with recurrence but also in those without them. These considerations imply that after colorectal cancer resection, tumor marker determination is of little if any value, since they may mislead a

correct diagnosis which has to be confirmed by imaging until more sensitive and specific tests are available.

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